Memantine, an NMDA Receptor Antagonist, Prevents Thyroxin-Induced Hypertension, but Not Cardiac Remodeling

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Steven Joseph Repas

The Ohio State University
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Project Advisor: Dr. Paul Janssen, Department of Physiology and Cell Biology

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Steven J. Repas[1,2]; BSc, Nancy S. Saad[1,2,3]; MSc, Paul M.L. Janssen[1,2¶]; PhD and Mohammad T. Elnakish[1,2,3¶]; PhD

[1]Department of Physiology and Cell Biology, College of Medicine, [2]Dorothy M. Davis Heart & Lung Research Institute, the Ohio State University, Columbus, OH, USA.

[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

[¶] Contributed equally

Address for correspondence:

Mohammad T. Elnakish, PhD
Department of Physiology and Cell Biology
304 Hamilton Hall, 1645 Neil Avenue,
Columbus, OH-43210, USA
Phone: 614-292-7450
Fax: 614-292-4888
Email: elnakish.1@osu.edu

Paul M.L. Janssen, PhD
Department of Physiology and Cell Biology
304 Hamilton Hall, 1645 Neil Avenue,
Columbus, OH-43210, USA
Phone: 614-247-7838
Fax: 614-292-4888
Email: janssen.10@osu.edu

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Abstract

Stimulation of glutamatergic tone has been causally linked to myocardial pathogenesis and amplified systemic blood pressure (BP). Memantine, a non-competitive *N*-Methyl-D-aspartate glutamatergic receptor (NMDA-R) antagonist, has been proposed to be an active cardioprotective drug. However, the efficacy of memantine and subsequently the possible involvement of the NMDA-R in the thyroxin (T4)-induced cardiovascular complications have never been investigated. We examined the effect of memantine (30 mg/kg/day/I.P) on the T4 (500 µg/kg/day/I.P)-provoked increase in mouse BP as well as cardiac hypertrophy and reformed reactions of the contractile myocardium both *in-vivo* and *ex-vivo* following 2 weeks of treatment. Memantine alone did not result in any cardiovascular pathology in mice. Instead, memantine significantly prevented the T4-triggered systemic hypertension. But, it did not reverse cardiac hypertrophy, coupled *in-vivo* left ventricular (LV) dysfunction or *ex-vivo* right ventricular (RV) papillary muscle contractile alterations of the T4-treated mice. Our results openly direct the cardiovascular safety and tolerability of memantine therapy. Yet, extra research is necessary to endorse these prospective advantageous outcomes. Also, we believe that this is the first study to inspect the possible role of NMDA-R in the T4-stimulated cardiovascular disorders and concluded that NMDA-R could play a key role in the T4-induced hypertension.

Key words: Memantine, Thyroxin, Hypertension

Background

Glutamatergic neurotransmission is a remarkable excitatory pathway in the central nervous system, where it regulates a broad diversity of neuronal activities based on the glutamate receptor type.[1] Generally, there are two different types of glutamate receptors, which are the ionotropic receptors (ligand-gated channels) and the metabotropic receptors (G protein-coupled receptors). The ionotropic receptors are differentiated into three subtypes in proportion to their explicit agonists: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPA-R), kainite receptor (KA-R) and NMDA-R.[1,2]

Although NMDA-R expression is well-known for the central nervous system, its expression has been also demonstrated in peripheral organs, including the cardiomyocytes[3,4] and endothelial cells.[5] Interestingly, NMDA-R activation has been causally associated with myocardial pathogenesis, such as heart failure,[6,7] ventricular arrhythmias[8] and atrial fibrillation,[9] in addition to systemic hypertension.[10] These cardiac pathologies evoked by the activation of NMDA-R were attributed to increased oxidative stress, calcium overload, cardiomyocyte apoptosis,[1] myocardial fibrosis[8] and stimulation of myocyte mitochondrial matrix metalloproteinase.[11]

Similar to the NMDA-R activation, increased thyroid hormones (TH), a well-documented modulator of cardiovascular function, can trigger heart failure,[12,13] ventricular arrhythmias,[14] atrial fibrillation,[15] as well as systemic hypertension.[16-18] Additionally, some analogous cardiac mechanisms were reported in the TH-induced cardiovascular pathology, counting the oxidative stress,[19, 20] cardiomyocyte apoptosis[21] and matrix metalloproteinase.[22]

Depending on these parallel outcomes, we hypothesized that NMDA-R may be involved in the TH-aggravated cardiac remodeling and hemodynamic changes. Thus, in the current study, this hypothesis was examined by exploring the effect of memantine, a non-competitive NMDA-R

antagonist, on the T4-driven increase in BP as well as cardiac hypertrophy and reformed reactions of the contractile myocardium both *in-vivo* and *ex-vivo* in mice.

Methods

Animals

Adult (9-12 months old) FVB/N male mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and kept at the Research Animal Facility of The Ohio State University for the whole duration of the experimental procedures. The experimental procedures and protocols used in this study were approved by the Animal Care and Use Committee of the Ohio State University, conforming to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (National Institutes of Health publication No. 85–23, revised 1996).

Thyroxin (T4) and Memantine HCL Treatments

T4, sodium-L-thyroxin, from Sigma-Aldrich (St. Louis, MO) was prepared as previously described[23] and injected intraperitoneally at a dose of 500 mg/kg/day for 2 weeks as we reported before.[24-26] Memantine HCL from Sigma-Aldrich (St. Louis, MO) was dissolved in normal saline and administered at a dose of 30 mg/kg/day by intraperitoneal injection prior to T4. Memantine dose was chosen based on a previous study showed that 30 mg/kg/day in mice constructs the 1 μM therapeutic steady-state plasma level as indicated by numerous preclinical and clinical studies.[27] Mice were divided into 4 groups based on treatment; vehicle (control group) (n = 16), memantine only (n =14), T4 only (n = 17) and memantine + T4 (n = 15). At the end of the treatment period, mice underwent BP measurements, echocardiography and electrocardiogram (ECG). Thereafter, mice were sacrificed, and hearts were removed and processed for further experiments.

Blood pressure (BP) measurements

BP was measured noninvasively in conscious untrained mice by the tail cuff method using a six-Channel CODA High Throughput Acquisition system (Kent Scientific Corporation, Torrington, CT, USA) as described before.[25] Each experimental session consisted of 10 acclimatization cycles followed by 10 BP measurements cycles. Only the accepted cycles as identified by the BP measurement software are included in the data analysis. The average of all accepted cycles from one session was used for systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure (MAP) in each mouse.

Echocardiography

The *in-vivo* LV dimension and contractile function in mice were evaluated by using a high-frequency ultrasound imaging system (VEVO 2100, Visual Sonics, Toronto, ON, Canada) as described before.[23-26] Mice were anesthetized with isoflurane at a concentration of 3 % and thereafter maintained at 1.5 % isoflurane via nasal prongs during the whole procedure. The measurements were taken from the parasternal short-axis view in M-mode to view the LV movement during systole and diastole corresponding to the electrocardiogram. All data and imaging were analyzed by the Visual Sonics Cardiac Measurements Package.

Electrocardiogram (ECG)

ECG parameters including heart rate (HR), PR, QRS, and QT intervals were recorded noninvasively in fully conscious and unrestrained mice using the ECGenie system (Mouse Specifics, Inc., MA). Mice were placed onto the recording platform for sufficient time (about 30 minutes) to acclimate and trigger recordings when their paws are in contact with the recording electrodes. All data were then analyzed by e-MOUSE, a Physiological Data Analysis and Database Portal, as we previously reported.[24,26]

Heart weight (HW), cardiac muscle preparation and experimental setup

First, mice were weighed, and five minutes after I.P heparin injection mice were euthanized by cervical dislocation. After bilateral thoracotomy, hearts were rapidly excised and placed in Krebs–Henseleit buffer containing (in mmol/L): 120 NaCl, 5 KCl, 2 $MgSO_4$, 1.2 $NaH2PO_4$, 20 $NaHCO_3$, 0.25 Ca^{2+}, and 10 glucose, equilibrated with 95% O_2- 5% CO_2, resulting in a *pH* of 7.4. Additionally, 20 mmol/L 2,3-butanedione monoxime (BDM) was added to the dissection buffer to prevent cutting injury.[24-26] Extra non-cardiac tissues, such as fat and pieces of lung, were carefully removed. After hearts were blotted gently on Kim wipes they rapidly transferred to a small weigh dish that contained clean oxygenated Krebs–Henseleit/BDM buffer that was tarred to zero on an electronic analytical balance to get the exact wet HW. HW/body weight (BW) ratios were then calculated and expressed as mg/g. Hearts were then carefully opened, repeatedly perfused with the same oxygenated Krebs–Henseleit/BDM buffer, and blood was thoroughly washed out. From the RV, uniform linear papillary muscles were carefully dissected. The dimensions of muscles were measured using a calibration reticule in the ocular of the dissection microscope (40x, resolution ~10 µm). The cross-sectional areas were calculated assuming ellipsoid cross-sectional shapes. There was no significant difference between average dimensions (width x thickness x length) of the control (0.25 ± 0.02 x 0.16 ± 0.01 x 1.02 ± 0.10 mm), memantine (0.25 ± 0.02 x 0.16 ± 0.01 x 0.80 ± 0.08 mm), T4 (0.30 ± 0.02 x 0.20 ± 0.02 x 0.88 ± 0.08 mm) and memantine +T4 (0.29 ± 0.02 x 0.19 ± 0.01 x 0.88 ± 0.06 mm). P values are 0.1219, 0.1097, and 0.3265, respectively.

With the use of the dissection microscope, muscles were mounted between basket-shaped extension of a force transducer (KG7, Scientific Instruments, Heidelberg, Germany) and a hook (valve end) connected to a micromanipulator as previously mentioned.[24-26] Muscles were

superfused with the same buffer at 37.5^{o}C as above (with the exception that BDM was omitted) and stimulated at 4 Hz. Extracellular Ca^{2+} concentration was raised to 2 mmol/L and muscles were allowed to stabilize for at least 30 minutes before the experimental protocol was initiated. The 4 Hz baseline was selected rather than a more physiological 12 Hz, as in our previous reports.[24-26] However, to study more physiological frequencies, 12 Hz contractions were also assessed, but only for brief periods. Generally, muscles were stretched to an optimal length where a small increase in length resulted in nearly equal increases in resting tension and active developed tension. This length was selected to be comparable to the maximally attained length *in-vivo* at the end of diastole.[28]

To obtain a broad scope of quantitative data to dissect contractile function and dysfunction, two of the three main mechanisms utilized *in-vivo* to physiologically modify force of contraction, frequency-dependent activation, and β-adrenergic stimulation were assessed in mouse papillary muscles under near physiological conditions as previously described.[24-26] This is because the third mechanism, which is the length-dependent activation (Frank-Starling mechanism) is well-sustained in the hearts of these T4-treated mice.[25] We assessed the effect of increasing stimulation frequencies between 4 and 14 Hz, spanning the entire *in-vivo* range of the mouse. At each frequency, forces were allowed to reach steady state before data were recorded. The effects of β-adrenergic stimulation were also assessed by a concentration–response curve with isoproterenol (10^{-9}–10^{-6} mol/L) at a baseline stimulation frequency of 4 Hz.

In all experiments performed peak isometric developed force (Fdev) was determined and normalized to the cross-sectional area of the muscle. Additionally, as a force-independent parameter of force decay kinetics, time to peak force (TTP), and time from peak force to 50% relaxation (RT50) were determined. Muscles with an initial Fdev or a Fdev after re-stabilization

following frequency-dependent activation < 5 mN/mm^2 were excluded from the analysis of all experimental parameters. Additionally, muscles that displayed arrhythmia early at the initial isoproterenol concentrations were excluded from the final isoproterenol-Fdev data analysis. Furthermore, muscles that exhibited arrhythmia after the FFR and before starting the isoproterenol experiments were not included either in the arrhythmia or in the final isoproterenol-Fdev data analysis (1 muscle from the control group and 1 from the T4 group).

Data analysis and statistics

Generally, a two-tailed value of $P \leq 0.05$ was considered statistically significant. Data are presented as mean ± SEM and were analyzed by either ordinary one-way analysis of variance (ANOVA) or repeated measures ANOVA followed by Tukey-Kramer's post-hoc multiple comparison test. Ordinary one-way ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption was tested using Kolmogorov and Smirnov test, which showed that all data sampled passed normality test. Also, it assumes that the data are sampled from populations with identical SDs and this assumption was tested using Bartlett test. The cases, in which Bartlett test suggested significant differences among the SDs of the groups Kruskal-Wallis test (nonparametric ANOVA) was applied followed by Dunn's post-hoc multiple comparison test. On the other hand, ordinary repeated measures ANOVA assumes effective matching amongst means. If the matching appears not to be effective, Friedman test (nonparametric repeated measures ANOVA) was applied followed by Dunn's post-hoc multiple comparison test.

Results

First, we did not observe a significant difference in the SBP (113.90 ± 3.96 mmHg), DBP (85.84 ± 4.30 mmHg) and MAP (94.83 ± 4.14 mmHg) of the mice treated only with memantine compared to the control (118.40 ± 2.23 mmHg), (94.24 ± 2.23 mmHg) and (101.91 ± 2.20 mmHg), respectively (**Figure 1A-C**). Conversely, the T4 treatment as expected[16-18,24-26] significantly increased the SBP (141.77 ± 3.05 mmHg; $p<0.001$), DBP (112.11 ± 3.06; mmHg $p<0.001$) and MAP (121.72 ± 3.05 mmHg; $p<0.001$) compared to both the control and the memantine only-treated mice (**Figure 1A-C**). Notably, concomitant administration of memantine with T4 significantly reduced the remarkably amplified SBP, DBP and MAP following T4 treatment to a level that was not significant compared to both the control and the memantine only-treated mice (119.79 ± 3.17 mmHg; $p<0.001$), (87.77 ± 3.27 mmHg; $p<0.001$) and (98.15 ± 3.20 mmHg; $p<0.001$), respectively (**Figure 1A-C**).

Echocardiography analysis illustrated that memantine treatment could not cause a significant change in the LV ejection fraction (EF) (66.23 ± 0.68 %) (**Figure 2A**) or the fractional shortening (FS) (36.07 ± 0.50 %) (**Figure 2B**) compared to those of the control (EF: 67.59 ± 0.87 % and FS: 37.23 ± 0.64 %). However, in agreement with our former outcomes[24-26] as well as with others' data,[12,13] the T4 treatment led to LV systolic dysfunction as revealed by significantly decreased EF (58.79 ± 1.40 %) (**Figure 2A**) and FS (30.99 ± 0.96 %) (**Figure 2B**) compared to those of both the control (EF: $p < 0.001$ and FS: $p < 0.001$) and the memantine only-treated mice (EF: $p < 0.01$ and FS: $p < 0.01$). Also, concomitant administration of memantine with T4 could not inhibit the T4-prompted reduction in either the EF or the FS and they were still significantly lower than those of the control (EF: 60.63 ± 1.32 %; $p < 0.01$ and FS: 32.31 ± 0.93 %; $p < 0.01$) (**Figure 2A, B**). Furthermore, echocardiographic assessment demonstrated that mice with

memantine alone had no significant changes in the LV mass (108.69 ± 4.01 mg) (**Figure 2C**) or the LV mass/BW ratio (3.75 ± 0.12 mg/g) (**Figure 2D**) compared to those of the control (LV mass: 106.24 ± 3.40 mg and LV mass/BW: 3.63 ± 0.09 mg/g). Nevertheless, the T4 treatment as we reported before[24-26] resulted in a marked increase in the LV mass (157.37 ± 5.82 mg; $p < 0.001$) (**Figure 2C**) and LV mass/BW ratio (5.09 ± 0.14mg/g; $p < 0.001$) (**Figure 2D**) compared to those of both the control and the memantine only-treated mice. Memantine along with T4 treatment could not significantly diminish such T4-accelerated increases in LV mass or LV mass/BW ratio and they were still significantly higher than those of the control and the memantine only-treaded mice (148.09 ± 5.15 mg; $p < 0.001$ and 5.18 ± 0.17 mg/g; $p < 0.001$, respectively) (**Figure 2C, D**). On the other hand, the ECG analysis showed no significant difference in any of the parameters that were measured (HR, PR, QRS and QT) among all groups, including the T4-treated mice as we formerly defined[24, 26] **(Table 1)**.

Evaluation of the BW using the Kruskal-Wallis test showed a P value of 0.0408, which is considered significant. However, the Dunn's Multiple Comparisons test demonstrated a non-significant difference amongst the groups: control (29.53 ± 0.30 g), memantine (28.92 ± 0.39 g), T4 (30.88 ± 0.59) and memantine + T4 (28.74 ± 0.63 g). Yet, there was a quite non-significant difference in the BW of the memantine ($P = 0.0815$) and the memantine + T4 ($P = 0.0968$) compared to the T4-treated mice (**Figure 3A**). Besides, there was no significant difference in the HW (136.69 ± 2.42 mg) or the HW/BW ratio (4.73 ± 0.05 mg/g) of the mice treated with memantine alone compared to those of the control mice (HW: 139.13 ± 2.10 mg and HW/BW: 4.71 ± 0.06 mg/g) (**Figure 3B**). Alternatively, T4 treatment significantly increased both HW (186.83 ± 3.71 mg: $p < 0.001$) and HW/BW ratio (6.06 ± 0.09: $p < 0.001$) compared to both the control and the memantine only-treated mice (**Figure 3C**), which confirms the development of

cardiac hypertrophy in harmony with our previous reports.[24-26] Memantine administration along with T4 treatment did not significantly decrease the T4-evoked increase in HW or HW/BW ratio and were still notably higher than those of the control and memantine-only treated mice (HW: 183.34 ± 5.30 mg; $p < 0.001$ and HW/BW: 6.40 ± 0.20 mg/g; $p < 0.001$) (**Figure 3B, C**).

Finally, under proximate physiological temperature and at a preload leading to sarcomere length around the *in-vivo* end-diastolic values of 2.2 μm,[28] the *ex-vivo* experiments exemplified that there is no significant difference in the Fdev of the RV papillary muscles amongst the groups ($p = 0.4684$) at a stimulation frequency of 4 Hz, including the T4 group resembling our earlier records (24-26): control: 26.0 ± 3.7 mN/mm^2, memantine: 22.6 ± 4.6 mN/mm^2,T4: 19.6 ± 1.9 mN/mm^2 and memantine + T4: 17.2 ± 3.0 mN/mm^2) (**Figure 4A**). Moreover, muscles from the control and the memantine only-treated mice exhibited similar contractile profile as indicated by comparable TTP (47.1 ± 1.2 ms vs. 46.3 ± 1.7 ms) (**Figure 4B**) and RT50 (24.6 ± 1.7 ms vs. 31.1 ± 1.8 ms), respectively (**Figure 4C**). Inversely, the muscles from the T4-treated mice demonstrated faster contraction and relaxation as we explained before[24-26] by significantly declining the TTP (39.1 ± 0.7 ms) and the RT50 (18.9 ± 0.8 ms) compared to those of the control (TTP; $p < 0.01$ and RT50; $p < 0.05$) and the memantine only group (TTP; $p < 0.05$ and RT50: $p < 0.001$) (**Figure 4B, C**). Additionally, concomitant administration of memantine with T4 was not able to significantly change the T4-induced decrease in the TTP (38.4 ± 1.1 ms) or the RT50 (19.3 ± 0.8 ms). The TTP of memantine +T4 group was still significantly lower than those of the control ($p < 0.001$) and the memantine only group ($p < 0.01$), However, the RT50 of the memantine + T4 group was only significant compared to the memantine only ($p< 0.001$), but not the control group ($p = 0.1558$) (**Figure 4B, C**).

Fdev was also assessed within the murine *in-vivo* physiological range (8–12 Hz) along with the baseline stimulation frequency of 4 Hz. Muscles from the T4-treated mice exhibited a significantly negative response to the increasing frequency at 10 Hz (versus memantine; $p < 0.05$), 12 Hz (versus control; $p < 0.05$) and 14 Hz (versus control; $p < 0.05$) as indicated by ordinary one-way ANOVA and there was no significant differences amongst the rest of the groups (**Figure 3A**). On the other hand, Friedman test of repeated measures ANOVA displayed a substantial influence for both the group ($P<0.001$) and frequency ($P<0.001$) variations on the Fdev. But, there was no considerable interaction between the 2 variants. Notably, the force frequency response in this study is somewhat different from our previous reports,[24-26] where the control mice (7 - 9 months) typically show a considerable positive response compared to the negative response that we demonstrated here (9 - 12 months). The most likely explanation is that the mouse's age seems to be a key factor. We have illustrated that isolated papillary muscles from the older wild-type FVB/N mice RV (12 - 14 months) exhibited a slightly negative force frequency response,[29] in addition to a similar response that we have recently discovered in another group of mice (9 - 10 months) (data not shown). The mice used in these studies are retired breeders and the exact age is not guaranteed.

Furthermore, the impact of the β-adrenergic stimulation was assessed by a concentration–response curve with isoproterenol (10^{-9}–10^{-6} mol/L) at a baseline stimulation frequency of 4 Hz. Under maximal β-adrenergic stimulation (1 μmol/L isoproterenol), there was no significant difference between muscle response of the control and the memantine only-treated mice. Nonetheless, Kruskal-Wallis test confirmed our preceding facts[24-26] and revealed that muscles from the T4-treated mice demonstrated a strikingly declined response versus those of the control ($P < 0.01$), but not the memantine only ($P = 0.0769$) group (**Figure 3B**). Memantine treatment could not prevent this dampened isoproterenol response in the muscles of the T4-treated mice and it

continue to be considerably reduced compared to the muscles of control ($p < 0.01$) and memantine only-treated mice ($P < 0.05$) (**Figure 3B**). Besides, repeated measures ANOVA presented a notable effect for the group ($P<0.001$) and the isoproterenol concentration ($P<0.001$) variations on the Fdev, along with a substantial interaction between the 2 variants ($P < 0.001$). Finally, at maximal β-adrenergic stimulation, muscles of the T4 as well as the memantine + T4 groups exhibited arrhythmia in undoubtedly higher number of muscles (8/13 and 8/11, respectively) compared to those of the control (3/13) and the memantine alone (2/12) (**Figure 3C**).

Discussion

Thyroid hormones affect the glutamatergic neurotransmission pathway in the central nervous system, where they have been anticipated as a key modulator of NMDA-R subunit expression in the hippocampus.[30] In addition, Losi et al. reported that thyroid hormones nongenomically regulate the activity of the NMDA-R and repressed the neuronal death driven by glutamate.[31] Moreover, it has been shown that hypothyroidism could protect from cerebral ischemia due to subsequent decrease in glutamate release.[32] However, the relation between this glutamatergic pathway and thyroid hormone in the peripheral tissue, in particular, the cardiovascular system, has never been exposed. Previous studies reported that NMDA-Rs are widely expressed in the hearts of human and rodent.[33,34] The NMDA-R expression has been also detected in the vasculature, including the aorta and pulmonary artery.[34] In the vein of thyroid hormone, NMDA-R activation has been linked to some kind of circulatory diseases, such as heart failure, cardiac arrhythmias and hypertension.[6-10] Therefore, it is rational to assume that the NMDA-R could be a prospective intervention target for the T4-induced cardiovascular changes and we believe that this is the first study to examine this hypothesis.

Memantine is a non-competitive NMDA-R antagonist[35] characterized by a fast-binding/dissociating activity, which allows for a fast course of action and less effect on physiologic functions compared to the other NMDA-R antagonists.[36,37] Hence, memantine has been stated to offer much promise in the treatment of neurodegenerative disorder,[38] and is presently used in moderate to severe Alzheimer's disease (AD) patient therapy in the US and Europe.[37,39] Memantine has been also shown to be a non-competitive antagonist of both the serotonin (5-HT3) receptors[40] and the nicotinic cholinergic receptors,[41] as well as a dopaminergic receptor agonist.[42]

Yet, when used in combination therapy, for example, its 5HT-3 antagonism could prevent the acetyl cholinesterase inhibitor gastrointestinal side effects.[37]

Memantine is a well-tolerated drug,[37] so far little is known about its cardiovascular effects.[43] Clinical trials have shown that hypertension is one of the memantine side effects occurred in > 2% of the patients at a larger rate compared to the placebo-treated patients, but, it did not reach significance.[37] In the current study, we showed that memantine has no significant effect on the mouse BP. In agreement with our results, memantine at a similar dose (32 mg/kg) did not significantly affect the rat BP as well[44] and similarly the AP-5, an NMDA-R antagonist resulted in a very slight change in the BP of the sham rat.[7]

In addition, a number of compromised cardiac upshots have been reported following memantine therapy. For instance, an increase in the deadly and non-deadly myocardial infarction in the users of memantine has been reported in a comparison of the users and non-users of memantine in two large population databases. Though, this might be partly because of the sicker individual selection for memantine treatment.[43,45] Also, concomitant administration of memantine with donepezil in AD patient was displayed to increase the ECG PR interval.[46] Besides, memantine has been conveyed to exacerbate the ECG corrected QT interval in the AD patients.[47,48] However, these patients have been diagnosed with severe heart diseases, in addition to old age and diabetes, which are known risk factors for the prolonged corrected QT interval.[47] Conversely, prolonged corrected QT interval was not recorded in the French PharmacoVigilance Database report regarding the patients receiving memantine therapy, and a small number of adverse cardiovascular effects, above all, cardiac bradycardia was documented.[49] Compatibly, the (+)-MK801, a noncompetitive NMDA receptor antagonist, was demonstrated to prompt bradycardia and positive inotropy in isolated rat cardiac tissues (Right atria, left atria and right ventricular strips).[3] Overall,

it has been suggested that memantine cardiovascular properties appear to be complicated, pretty indistinct and require further experimental and clinical studies.[49] Here, we showed that memantine could not induce any considerable alteration in the cardiac structure and function as evidenced by the typical HW, *in-vivo* echocardiographic LV structure/function, ECG parameters (HR, PR, QT, and QRS) and ex-*vivo* RV contractile performance following memantine treatment in comparison to the control mice. Merely, memantine prolonged the relaxation time (RT50) of the contractile RV muscles, however, it did not reach significance ($P = 0.1994$; Kruskal-Wallis test). Theses valuable endings have been observed in the mice treated not only with the memantine alone, but also in combination with the T4 treatment, which resulted in numerous cardiac variations that were sufficient to increase the risk for cardiac mortality, even so, it did not befall. These data openly direct the cardiovascular safety and tolerability of memantine therapy. Yet, extra research is necessary to endorse these prospective advantageous outcomes.

On the other side, memantine has shown some cardiac therapeutic effects. D'Amico et al. conveyed that NMDA/non-NMDA inotropic excitatory amino acid antagonists, including memantine, are effective in preventing the reperfusion-induced, but not the ischemia-induced cardiac arrhythmia and mortality in rats.[50] Additionally, it has been reported that memantine could prevent the reduction in the rat LV cardiomyocyte nuclear size induced by cold stress, which has been suggested to either initiate or increase the rodent cardiovascular dysfunction,[51,52] concluding that memantine might be a promising cardioprotective medication.[51] Actually, NMDA-R has been shown to play a key role in the induction of several heart failure/decreased cardiac contractility related pathways, such as the sympathoexcitation,[6,7] oxidative stress, calcium overload, cardiomyocyte apoptosis,[1,53] myocardial fibrosis[8] and stimulation of myocyte mitochondrial matrix metalloproteinase.[11] Even though T4-induced cardiac remodeling has been referred to

similar mechanisms, including the sympathetic nervous system,[54] oxidative stress,[19,20] cardiomyocyte apoptosis[21] and matrix metalloproteinase,[22] memantine could not inhibit any of the T4-induced cardiac pathological manifestations in the current study. Furthermore, NMDA-R-associated reduction in the cardiomyocyte contractility has been reported to be linked to prolongation in the TTP and RT[11,53] in contrast to the T4-evoked reduction in both parameters in the RV muscles of this model.

Prominently, molecular,[7] immunocytochemical[55] and biochemical[56] records have displayed that the NMDA-R expression could be adapted by alterations in the systemic BP. NMDA-R has been reported to markedly contribute to the hypertension prompted by raised sympathoexcitation associated with the left coronary ligation-induced heart failure in rats,[7] angiotensin II[57] and carotid clamping in the rostral ventrolateral medulla of anesthetized rats.[58] In consistency with these outcomes, memantine, an NMDA-R blocker, significantly reduced the T4-induced hypertension in our model. Interestingly, a recent study revealed that the glutamatergic neurotransmission in the rostral ventrolateral medulla of conscious rats depends on the nitric oxide (NO)-NMDA-R pathway excitatory effects to control the systemic BP.[10] Indeed, we[25] and others[18] have shown that the NO synthase (NOS) pathway is a key player in the T4-evoked hypertension. Generally, the activation of the NOS has been reported in the hyperdynamic circulation following hyperthyroidism. While endothelial NOS (eNOS) and inducible NOS (iNOS) could have a homeostatic role, neuronal NOS (nNOS) was projected as the crucial factor in the hyperthyroidism-induced hypertension in rats.[16-18] Notably, the nNOS activation with the subsequent NO production has been reported as a strategic NMDA-R signaling transducer[59] and has been involved in the regulation of BP and neurogenic hypertension as well.[60] Based on these findings, we strongly believe that the inhibitory effects of memantine on the NO-NMDA-R

excitatory pathway could potentially be the main mechanism resulted in the significant prevention of the T4-induced hypertension in this study. However, future investigation for further confirmation is warranted.

Conclusions:

Overall, our results openly direct the cardiovascular safety and tolerability of memantine therapy. Yet, extra research is necessary to endorse these prospective advantageous outcomes. Also, we believe that this is the first study to inspect the possible role of NMDA-R in the T4-stimulated circulatory disorders. Finally, our data show for the first time that NMDA-R could play a key role in the T4-induced hypertension, but not cardiac remodeling.

References:

1. Gao X., Xu X., Pang J., Zhang C., Ding J., Peng X., Liu Y. and Cao J. NMDA receptor activation induces mitochondrial dysfunction, oxidative stress and apoptosis in cultured neonatal rat cardiomyocytes. Physiological research. 2007; 56(5):559.
2. Meldrum B.S. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. The Journal of nutrition. 2000; 130(4):1007S-1015S.
3. Huang C.-F. and Su M.-J. Positive inotropic action of NMDA receptor antagonist (+)-MK801 in rat heart. Journal of biomedical science. 1999; 6(6):387-398.
4. Krainc D., Bai G., Okamoto S.-i., Carles M., Kusiak J.W., Brent R.N. and Lipton S.A. Synergistic Activation of theN-Methyl-D-aspartate Receptor Subunit 1 Promoter by Myocyte Enhancer Factor 2C and Sp1. Journal of Biological Chemistry.1998; 273(40):26218-26224.
5. Qureshi I., Chen H., Brown A.T., Fitzgerald R., Zhang X., Breckenridge J., Kazi R., Crocker A.J., Stühlingexsr M.C. and Lin K. Homocysteine-induced vascular dysregulation is mediated by the NMDA receptor. Vascular Medicine.2005; 10(3):215-223.
6. Sharma N.M., Cunningham C.J., Zheng H., Liu X. and Patel K.P. Hypoxia-Inducible Factor-1α Mediates Increased Sympathoexcitation via Glutamatergic N-Methyl-d-Aspartate Receptors in the Paraventricular Nucleus of Rats With Chronic Heart Failure Circulation: Heart Failure. 2016; 9(11):e003423.
7. Li Y.-F., Cornish K.G. and Patel K.P. Alteration of NMDA NR1 receptors within the paraventricular nucleus of hypothalamus in rats with heart failure. Circulation research. 2003; 93(10):990-997.
8. Shi S., Liu T., Li Y., Qin M., Tang Y., Shen J.Y., Liang J., Yang B. and Huang C. Chronic N-Methyl-d-Aspartate Receptor Activation Induces Cardiac Electrical Remodeling and Increases Susceptibility to Ventricular Arrhythmias. Pacing and Clinical Electrophysiology. 2014; 37(10):1367-1377.
9. Shi S., Liu T., Wang D., Zhang Y., Liang J., Yang B. and Hu D. Activation of N-methyl-d-aspartate receptors reduces heart rate variability and facilitates atrial fibrillation in rats. Europace. 2016; euw086.
10. Machado N.L., Silva F.C., Chianca D.A. and de Menezes R.C. Nitric oxide modulates blood pressure through NMDA receptors in the rostral ventrolateral medulla of conscious rats. Brain research. 2016; 1643:159-167.
11. Moshal K.S., Tipparaju S.M., Vacek T.P., Kumar M., Singh M., Frank I.E., Patibandla P.K., Tyagi N., Rai J. and Metreveli N. Mitochondrial matrix metalloproteinase activation decreases myocyte contractility in hyperhomocysteinemia. American Journal of Physiology-Heart and Circulatory Physiology. 2008; 295(2):H890-H897.
12. Kuzman J.A., Thomas T.A., Vogelsang K.A., Said S., Anderson B.E. and Gerdes A.M. Effects of induced hyperthyroidism in normal and cardiomyopathic hamsters. Journal of Applied Physiology. 2005; 99(4):1428-1433.
13. Hübner N.S., Merkle A., Jung B., Elverfeldt D. and Harsan L.A. Analysis of left ventricular function of the mouse heart during experimentally induced hyperthyroidism and recovery. NMR in biomedicine. 2015; 28(1):116-123.
14. Lin H., Mitasikova M., Dlugosova K., Okruhlicova L., Imanaga I., Ogawa K., Weismann P. and Tribulova N. Thyroid hormones suppress epsilon-PKC signalling, down-regulate

connexin-43 and increase lethal arrhythmia susceptibility in non-diabetic and diabetic rat hearts. J Physiol Pharmacol. 2008; 59(2):271-285.
15. Hu Y., Jones S.P. and Dillmann W.H. Effects of hyperthyroidism on delayed rectifier K+ currents in left and right murine atria. American Journal of Physiology-Heart and Circulatory Physiology. 2005; 58(4):1448-1455.
16. Rodríguez-Gómez I., Sainz J., Wangensteen R., Moreno J.M., Duarte J., Osuna A. and Vargas F. Increased pressor sensitivity to chronic nitric oxide deficiency in hyperthyroid rats. Hypertension. 2003; 42(2):220-225.
17. Rodríguez-Gómez I., Wangensteen R., Moreno J.M., Chamorro V., Osuna A. and Vargas F. Effects of chronic inhibition of inducible nitric oxide synthase in hyperthyroid rats. American Journal of Physiology-Endocrinology and Metabolism. 2005; 288(6):E1252-E1257.
18. Wangensteen R., Rodríguez-Gómez I., Moreno J.M., Álvarez-Guerra M., Osuna A. and Vargas F. Effects of chronic treatment with 7-nitroindazole in hyperthyroid rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2006; 291(5):R1376-R1382.
19. Gredilla R., Barja G. and López-Torres M. Thyroid hormone-induced oxidative damage on lipids, glutathione and DNA in the mouse heart. Free radical research. 2001; 35(4):417-425.
20. Araujo A., Schenkel P., Enzveiler A., Fernandes T., Partata W., Llesuy S., Ribeiro M., Khaper N., Singal P. and Bello-Klein A. The role of redox signaling in cardiac hypertrophy induced by experimental hyperthyroidism. Journal of Molecular Endocrinology. 2008; 41(6):423-430.
21. Wang Y.-Y., Jiao B., Guo W.-G., Che H.-L. and Yu Z.-B. Excessive thyroxine enhances susceptibility to apoptosis and decreases contractility of cardiomyocytes. Molecular and Cellular Endocrinology. 2010; 320(1):67-75.
22. Roy S.G., Mishra S., Ghosh G. and Bandyopadhyay A. Thyroid hormone induces myocardial matrix degradation by activating matrix metalloproteinase-1. Matrix Biology. 2007; 26(4):269-279.
23. Elnakish M.T., Hassona M.D., Alhaj M.A., Moldovan L., Janssen P.M., Khan M. and Hassanain H.H. Rac-induced left ventricular dilation in thyroxin-treated ZmRacD transgenic mice: role of cardiomyocyte apoptosis and myocardial fibrosis. PloS one. 2012; 7(8):e42500.
24. Elnakish M.T., Moldovan L., Khan M., Hassanain H.H. and Janssen P.M. Myocardial Rac1 exhibits partial involvement in thyroxin-induced cardiomyocyte hypertrophy and its inhibition is not sufficient to improve cardiac dysfunction or contractile abnormalities in mouse papillary muscles. Journal of cardiovascular pharmacology. 2013; 61(6):536-544.
25. Elnakish M.T., Schultz E.J., Gearinger R.L., Saad N.S., Rastogi N., Ahmed A.A., Mohler P.J. and Janssen P.M. Differential involvement of various sources of reactive oxygen species in thyroxin-induced hemodynamic changes and contractile dysfunction of the heart and diaphragm muscles. Free Radical Biology and Medicine. 2015; 83:252-261.
26. Saad N.S., Floyd K., Ahmed A.A., Mohler P.J., Janssen P.M. and Elnakish M.T. The Effect of Sorafenib, Tadalafil and Macitentan Treatments on Thyroxin-Induced Hemodynamic Changes and Cardiac Abnormalities. PloS one. 2016; 11(4):e0153694.

27. Minkeviciene R., Banerjee P. and Tanila H. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. Journal of Pharmacology and Experimental Therapeutics. 2004; 311(2):677-682.
28. Rodriguez E.K., Hunter W.C., Royce M.J., Leppo M.K., Douglas A.S. and Weisman H.F. A method to reconstruct myocardial sarcomere lengths and orientations at transmural sites in beating canine hearts. American Journal of Physiology-Heart and Circulatory Physiology. 1992; 263(1):H293-H306.
29. Elnakish M.T., Hassanain H.H. and Janssen P.M. Vascular Remodeling–Associated Hypertension Leads to Left Ventricular Hypertrophy and Contractile Dysfunction in Profilin-1 Transgenic Mice. Journal of cardiovascular pharmacology. 2012; 60(6):544-552.
30. Lee P., Brady D. and Koenig J. Thyroid Hormone Regulation of N-Methyl-d-Aspartic Acid Receptor Subunit mRNA Expression in Adult Brain. Journal of neuroendocrinology. 2003; 15(1):87-92.
31. Losi G., Garzon G. and Puia G. Nongenomic regulation of glutamatergic neurotransmission in hippocampus by thyroid hormones. Neuroscience. 2008; 151(1):155-163.
32. Shuaib A., Ijaz S., Hemmings S., Galazka P., Ishaqzay R., Liu L., Ravindran J. and Miyashita H. Decreased glutamate release during hypothyroidism may contribute to protection in cerebral ischemia. Experimental neurology. 1994; 128(2):260-265.
33. Gill S., Veinot J., Kavanagh M. and Pulido O. Human heart glutamate receptors—implications for toxicology, food safety, and drug discovery. Toxicologic Pathology. 2007; 35(3):411-417.
34. Leung J.C., Travis B.R., Verlander J.W., Sandhu S.K., Yang S.-G., Zea A.H., Weiner I.D. and Silverstein D.M. Expression and developmental regulation of the NMDA receptor subunits in the kidney and cardiovascular system. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2002; 283(4):R964-R971.
35. Kornhuber J., Bormann J., Retz W., Hübers M. and Riederer P. Memantine displaces [3H] MK-801 at therapeutic concentrations in postmortem human frontal cortex. European journal of pharmacology. 1989; 166(3):589-590.
36. Chen H. and Lipton S.A. Mechanism of memantine block of NMDA-activated channels in rat retinal ganglion cells: uncompetitive antagonism. The journal of physiology. 1997; 499(Pt 1):27.
37. Thomas S.J. and Grossberg G.T. Memantine: a review of studies into its safety and efficacy in treating Alzheimer's disease and other dementias. Clin Interv Aging. 2009; 4:367-377.
38. Chen H., Pellegrini J., Aggarwal S., Lei S.Z., Warach S., Jensen F.E. and Lipton S. Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. Journal of Neuroscience. 1992; 12(11):4427-4436.
39. Reisberg B., Doody R., Stöffler A., Schmitt F., Ferris S. and Möbius H.J. Memantine in moderate-to-severe Alzheimer's disease. New England Journal of Medicine. 2003; 348(14):1333-1341.
40. Rammes G., Rupprecht R., Ferrari U., Zieglgänsberger W. and Parsons C. The N-methyl-D-aspartate receptor channel blockers memantine, MRZ 2/579 and other amino-alkyl-cyclohexanes antagonise 5-HT 3 receptor currents in cultured HEK-293 and N1E-115 cell systems in a non-competitive manner. Neuroscience letters. 2001; 306(1):81-84.

41. Buisson B. and Bertrand D. Open-channel blockers at the human α4β2 neuronal nicotinic acetylcholine receptor. Molecular pharmacology. 1998; 53(3):555-563.
42. Seeman P., Caruso C. and Lasaga M. Memantine agonist action at dopamine D2High receptors. Synapse. 2008; 62(2):149-153.
43. Howes L.G. Cardiovascular effects of drugs used to treat Alzheimer's disease. Drug safety. 2014; 37(6):391-395.
44. Herrero J.F., Headley P.M. and Parsons C.G. Memantine selectively depresses NMDA receptor-mediated responses of rat spinal neurones in vivo. Neuroscience letters. 1994; 165(1):37-40.
45. Fosbøl E.L., Peterson E.D., Holm E., Gislason G.H., Zhang Y., Curtis L.H., Køber L., Iwata I., Torp-Pedersen C. and Setoguchi S. Comparative Cardiovascular Safety of Dementia Medications: A Cross-National Study. Journal of the American Geriatrics Society. 2012; 60(12):2283-2289.
46. Igeta H., Suzuki Y., Motegi T., Sasaki A., Yokoyama Y. and Someya T. Deterioration in donepezil-induced PR prolongation after a coadministration of memantine in a patient with Alzheimer's disease. General hospital psychiatry. 2013; 35(6):680. e689-680. e610.
47. Kajitani K., Yanagimoto K., Monji A. and Maruyama T. Memantine Exacerbates Corrected QT Interval Prolongation in Alzheimer's Disease: A Case Report from an Unintentional Rechallenge. Journal of the American Geriatrics Society. 2016; 64(1):232-233.
48. Takehara H., Suzuki Y. and Someya T. QT prolongation associated with memantine in Alzheimer's disease. Psychiatry and clinical neurosciences. 2015; 69(4):239-240.
49. Gallini A., Sommet A. and Montastruc J.L. Does memantine induce bradycardia? A study in the French PharmacoVigilance Database. Pharmacoepidemiology and drug safety. 2008; 17(9):877-881.
50. D'Amico M., Di Filippo C., Rossi F. and Rossi F. Arrhythmias induced by myocardial ischaemia-reperfusion are sensitive to ionotropic excitatory amino acid receptor antagonists. European journal of pharmacology. 1999; 366(2):167-174.
51. Meneghini A., Ferreira C., Abreu L.C.d., Valenti V.E., Ferreira M., F Filho C. and Murad N. Memantine prevents cardiomyocytes nuclear size reduction in the left ventricle of rats exposed to cold stress. Clinics. 2009; 64(9):921-926.
52. Matsuoka N., Arakawa H., Kodama H. and Yamaguchi I. Characterization of stress-induced sudden death in cardiomyopathic hamsters. Journal of Pharmacology and Experimental Therapeutics. 1998; 284(1):125-135.
53. Moshal K.S., Kumar M., Tyagi N., Mishra P.K., Metreveli N., Rodriguez W.E. and Tyagi S.C. Restoration of contractility in hyperhomocysteinemia by cardiac-specific deletion of NMDA-R1. American Journal of Physiology-Heart and Circulatory Physiology. 2009; 296(3):H887.
54. Hu L.W., Benvenuti L.A., Liberti E.A., Carneiro-Ramos M.S. and Barreto-Chaves M.L.M. Thyroxine-induced cardiac hypertrophy: influence of adrenergic nervous system versus renin-angiotensin system on myocyte remodeling. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2003; 285(6):R1473-R1480.
55. Marques-Lopes J., Kempen T., Waters E.M., Pickel V.M., Iadecola C. and Milner T.A. Slow-pressor angiotensin II hypertension and concomitant dendritic NMDA receptor trafficking in estrogen receptor β–containing neurons of the mouse hypothalamic

paraventricular nucleus are sex and age dependent. Journal of Comparative Neurology. 2014; 522(13):3075-3090.
56. Li D.-P., Byan H.S. and Pan H.-L. Switch to glutamate receptor 2-lacking AMPA receptors increases neuronal excitability in hypothalamus and sympathetic drive in hypertension. Journal of Neuroscience. 2012; 32(1):372-380.
57. Glass M.J., Wang G., Coleman C.G., Chan J., Ogorodnik E., Van Kempen T.A., Milner T.A., Butler S.D., Young C.N. and Davisson R.L. NMDA receptor plasticity in the hypothalamic paraventricular nucleus contributes to the elevated blood pressure produced by angiotensin II. Journal of Neuroscience. 2015; 35(26):9558-9567.
58. Kao M.-C., Lee H., Chai C. and Wang Y. NMDA antagonists attenuate hypertension induced by carotid clamping in the rostral ventrolateral medulla of rats. Brain research. 1991; 549(1):83-89.
59. Garthwaite J. Concepts of neural nitric oxide-mediated transmission. European Journal of Neuroscience. 2008; 27(11):2783-2802.
60. Wang Y. and Golledge J. Neuronal nitric oxide synthase and sympathetic nerve activity in neurovascular and metabolic systems. Current neurovascular research. 2013; 10(1):81-89.

Figure Legends

Figure 1. Blood pressure (BP) measurements of mice. (**A**) SBP, systolic blood pressure; (**B**) DPB, diastolic blood pressure; (**C**) MAP, mean arterial pressure. Control; n = 16, Memantine; n = 14, Thyroxin (T4); n = 17, Memantine + T4; n = 15. #, indicates a significant change compared to the T4 group; +, indicates a significant change compared to both the Control and the Memantine groups. One-way ANOVA followed by Tukey-Kramer's post-hoc multiple comparison test.

Figure 2. **Echocardiography Analysis of Mouse Hearts**. (**A**) Ejection fraction (EF), (**B**) Fractional shortening (FS), (**C**) left ventricle (LV) mass and (**D**) LV mass/body weight (BW) ratio. Control; n = 16, Memantine; n = 14, Thyroxin (T4); n = 17, Memantine + T4; n = 15. *, indicates a significant change compared to the control; +, indicates a significant change compared to both the Control and the Memantine groups. EF and FS; Kruskal Wallis test followed by Dunn's post-hoc multiple comparison test. LV mass and LV mass/BW ratio; one-way ANOVA followed by Tukey-Kramer's post-hoc multiple comparison test.

Figure 3. Morphological data. (**A**) Body weight (BW), (**B**) heart weight (HW) and (**C**) HW/BW. Control; n = 15, Memantine; n = 14, Thyroxin (T4); n = 17, Memantine + T4; n = 14. +, indicates a significant change compared to both the Control and the Memantine groups. Kruskal Wallis test followed by Dunn's post-hoc multiple comparison test.

Figure 4. Contractile profile of isolated right-ventricular papillary muscles. (**A**) Mean active isometric developed force (F_{dev}), (**B**) corresponding time to peak (TTP), and (**C**) 50% relaxation time (RT_{50}) at 4 Hz stimulation frequency and 2 mmol/L Ca^{2+}. Control; n = 14, Memantine; n = 12, Thyroxin (T4); n = 14, Memantine + T4; n = 11. !, indicates a significant change compared to

the Memantine group. +, indicates a significant change compared to both the Control and the Memantine groups. Kruskal Wallis test followed by Dunn's post-hoc multiple comparison test.

Figure 5. (**A**) **Force-frequency relationship of isolated right ventricular papillary muscles**. Peak isometric developed force (Fdev) values are expressed as a fraction of its corresponding Fdev at the basal frequency of 4 Hz and presented as mean ± SEM. Control; n = 14, Memantine; n = 12, Thyroxin (T4); n = 14, Memantine + T4; n = 11. One-way ANOVA followed by Tukey-Kramer's post-hoc multiple comparison test. (**B**) **β–adrenergic stimulation of isolated right ventricular papillary muscles**. Fdev values following β–adrenergic stimulation are expressed as ΔFdev, which is the change in the Fdev at (10^{-6} mol/l) isoproterenol in regard to its corresponding Fdev at the basal condition. Control; n = 13, Memantine; n = 11, Thyroxin (T4); n = 13, Memantine + T4; n = 8. Kruskal Wallis test followed by Dunn's post-hoc multiple comparison test at all isoproterenol concentrations, except the 10^{-8} M (One-way ANOVA followed by Tukey-Kramer's post-hoc multiple comparison test). (**C**) **Arrhythmic activity following β-adrenergic stimulation**. #, indicates a significant change compared to the T4 group. ϖ, indicates a significant change compared to both the T4 and the (Memantine + T4) groups. **τ**, indicates a significant change compared to the (Memantine + T4) group.⊗, indicates a significant change compared to both the group and frequency variation (Friedman nonparametric repeated measures ANOVA followed by Dunn's post-hoc multiple comparison test) or the group and the isoproterenol concentration variations (repeated measures ANOVA followed by Tukey-Kramer's post-hoc multiple comparison test). Error bars of the 2 upper groups; Control (⌶) and Memantine (I). Significance marks are directly above the error bar of the statistically significant group.